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Alteration of butyrylcholinesterase level in cholelithiasis patients after laparoscopic cholecystectomy: Do butyrylcholinesterase levels affect lipid metabolism?

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Abstract
Aim: Cholelithiasis (gallbladder stone) is a disease with a high incidence worldwide. The disease is multifactorial and various factors such as gender, age, obesity and use of oral contraceptives are held responsible for the development of the disease. In addition, lipid disorder is observed in more than 50% of patients with cholelithiasis. Laparoscopic cholecystectomy (LC) is one of the most frequently used surgical methods in the treatment of cholelithiasis, and there are data indicating that lipid profile changes and metabolic syndrome (MetS) develop after the operation. In this study, we aimed to investigate whether there is a change in lipid profile and Butyrylcholinesterase (BChE) activity, which affects lipid metabolism, in cholelithiasis patients after LC.
Material and Methods: In our study, 31 patients (obese and non-obese) who applied to the general surgery clinic of Nigde Omer Halisdemir University Training and Research Hospital were included. Blood samples were taken from the patients before LC and 6 months after the operation, and the lipid levels and BChE enzyme activity were examined using spectrophotometric method.
Results: According to the results, it was determined that the total cholesterol (p=0.015) and LDL (p=0.010) levels significantly decreased after LC, while no significant difference was found in the other parameters examined (p>0.05). In addition, it was observed that there was no significant correlation between the lipid profile examined both before and after LC and BChE activity level.
Discussion: According to the data obtained, the significant decrease in LDL and total cholesterol after LC indicates that this operation causes a positive change at least within a period of 6 months in the lipid profile of patients.

Keywords
Butyrylcholinesterase; Laparoscopic Cholecystectomy; Lipid Metabolism; Cholelithiasis; Lipid Profile

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Introduction

Cholelithiasis (gallbladder stone) can develop in individuals due to obesity, diabetes, and hyperlipidemia, increased bile cholesterol content with the effect of estrogen, bacterial infections and liver diseases [1]. Both environmental and genetic factors are effective in the pathogenesis of the disease [2]. Cholecystectomy is the most effective and preferred treatment method for cholelithiasis today [3]. Cholecystectomy can be performed in two different ways: open cholecystectomy and laparoscopic cholecystectomy (LC). Among these two operations, LC is the most frequently used method worldwide and attracts attention as one of the most popular surgical applications today [2, 4]. Applied for a long time, cholecystectomy has been accepted as a safe procedure with no harmful effects on individuals, and has become the preferred operation for symptomatic cholelithiasis today. At the same time, LC is a surgical procedure with minimal mortality and morbidity rates and ideal results in the short and long term [5]. In addition, although LC seems to be an intervention with the lowest risk and complications for cholelithiasis patients, recent studies have reported that this surgical procedure increases the risk of metabolic syndrome (MetS), which has become a common public health problem in populations today [1, 6]. MetS is defined as the combination of a number of risk factors such as central fat, high blood pressure, low HDL, high triacylglycerol (TAG) and hyperglycemia [7, 8]. It has been determined that the rate of development of various complications such as dyslipidemia, non-alcoholic fatty liver disease and hyperglycemia was observed in patients who underwent cholecystectomy [9]. These complications are also closely associated with MetS [10]. In addition, a significant increase in body mass index (BMI) and waist circumference has been reported in patients who underwent cholecystectomy. These data also support the thoughts that the risk of developing MetS after cholecystectomy is increased. It is understood that many of the complications that develop after cholecystectomy related to MetS are associated with lipid metabolism [11]. Since the gallbladder is an important organ in the digestion of lipids, it may be possible that there will be significant changes in the level and metabolism of lipids after cholecystectomy. In addition, in some other studies, it has been observed that TC, HDL, LDL, VLDL and TG levels significantly changed after cholecystectomy [12, 13]. Human butyrylcholinesterase enzyme is an enzyme in glycoprotein structure, which is mainly synthesized in the liver (BChE, EC.3.1.1.8). Enzyme activity is also found in the brain, pancreas and plasma, as well as in the liver. This enzyme is also called pseudo or non-neuronal cholinesterase [14, 15]. The fact that BChE is particularly effective on hydrophilic and hydrophobic cholinesterase has led to a focus on the pharmacological and toxicological functions of the enzyme. The enzyme provides hydrolysis of therapeutic and/or addictive drugs containing many esters such as succinylcholine, aspirin, irinotecan, heroin, and cocaine [16]. In addition, many clinical studies have revealed that varying serum BChE activity levels were associated with diseases such as obesity, impaired lipid metabolism, type 2 diabetes, and cardiovascular diseases, hypertension, hyperlipidemia, and hyperthyroidism [17-19]. Apart from all these, in a limited number of studies, a significant relationship has been found between BChE activity and LDL and VLDL levels [20]. In addition, it has been revealed that BChE significantly increases lipid metabolism and adipose tissue lipid deposits; however, the mechanism of these effects of BChE has not been elucidated in all aspects [21]. In this study, we aimed to examine whether there is a change in lipid profile and BChE activity levels after LC and to determine whether there is a correlation between lipid profile and BChE enzyme activity on obese and non-obese individuals.

Material and Methods

Composition of the Study Group

The study group composed of 31 volunteers (obese and non-obese) who applied to the general surgery clinic of Nigde Omer Halisdemir University Training and Research Hospital with complaints of abdominal pain, pain reaching the right shoulder, abdominal swelling, and who diagnosed with symptomatic cholelithiasis by ultrasonography.

Inclusion Criteria

The following patients were included in the study

- Non-obese (BMI<30 kg / m2)
- Obese (BMI=30-39.9 kg / m2)
- Diagnosis of symptomatic cholelithiasis

Exclusion Criteria were as follows:

- Hypertension,
- Morbid obesity (BMI> 40 kg / m2)
- Pancreatitis,
- Anemia,
- Nephrotic syndrome,
- Coronary heart disease,
- Pregnancy
- Any hormonal disease
- Receiving antihyperlipidemic therapy,
- Any previous bowel surgery

Prior to the study, approval was obtained from the Ethics Committee of Nigde Omer Halisdemir University Non-Interventional Clinical Research (No. 2021/34)

Collection of Blood Samples

After each of the individuals included in the patient group was informed in detail, and read and signed the informed consent form, blood samples were taken into 5 mL sterile biochemistry tubes with gel before and 6 months after the operation. After the blood samples taken from the patients were centrifuged at 4000 rpm for 10 minutes, their serum was separated and stored at -80 oC until the study day.

Measurement of HDL, TG, TC, LDL, BChE Levels

Serum samples obtained from the patients before the study day were removed from -80 degrees and kept at -40 oC for a while, then at -20 oC and then at 2-8 oC, and then they were taken to room temperature and prepared for the study. Lipid profile (HDL, Triglyceride, Total cholesterol, LDL) levels of patient serum samples were measured with the Roche Cobas c501 device by spectrophotometric method. According to the lipid profile results obtained, the LDL value was calculated according to the Friedewald formula. BChE enzyme levels were measured with the colorimetric method in Roche Cobas c501 device. In the colorimetric method, cholinesterase catalyzes the hydrolysis of butyrylthiocholine to thiocholine and butyrate. Thiocholine suddenly reduces the yellow hexacyanoferrate (III)

to an almost colorless hexacyanoferrate (II). This color reduction is measured photometrically. Analytical performances of the method for BChE were determined as intra-day precision [1st sample (5916 ± 28, CV = 0.5%) 2nd sample (7313 ± 38, CV = 0.5%)] and inter-day precision [3rd sample (1002 ± 26, CV = 2.6%) 4th sample (6683 ± 74, CV = 1.1%)]. The measurement on the device was calculated as U/L. The reference range of BChE enzyme levels for both male and female gender was determined by the manufacturer as 4260-12920 U/L.

Statistical Analysis:

The SPSS-15 software for Windows was used for statistical analysis. Analysis of parameters showing normal distribution was performed with the Paired-Samples T- test used for the dependent two-group analysis, and analysis of parameters not showing a normal distribution was performed with the Wilcoxon test, and the Mann-Whitney u test used for the dependent two-group analysis. Correlation analyses of the groups were calculated using Pearson's correlation for values with normal distribution and Spearman's correlation for parameters that do not show a normal distribution. Alpha significance level was taken as <0.05.

Results

As presented in Table 1, there were a total of 31 cholelithiasis patients in our study group, 8 males (26%), 23 females (74%). The mean age of the patients was 47.8 ± 2.3 years. When the study group is grouped in terms of BMI, it consists of 18 obese (58%) and 13 non-obese (42%) individuals.

According to the data we obtained, a statistically significant difference was found between cholesterol (p=0.015) and LDL (p=0.010) values before and after LC. However, no statistically significant difference was found in the values of BChE (p=0.215), TG (p=0.289), HDL (p=0.315), fasting blood glucose (p=0.651), creatinine (p = 0.580), total protein (p=0.155) and albumin (p=0.101) (Table 2).

According to the correlation test results, a significant positive correlation was found between albumin and HDL levels measured after LC (p=0.049; r:0.357). In addition, a weak positive correlation was analyzed for these two parameters, although not significant (p=0.07; r=0.376). However, there was no statistically significant correlation between other parameters examined before and after LC (p>0.05). When the study group was classified into two subgroups as obese (n=18) and non-obese (n=13) and evaluated in terms of the measured parameters, it was found that the LDL level after LC significantly decreased in both obese and non-obese groups (p<0.05), (Figure 1). No significant difference was observed in terms of other parameters examined (p>0.05), (Figure 1, 2)

Finally, when the correlations between the parameters measured before and after LC of the obese group and the non-obese group were analyzed, a weak negative correlation (p=0.039, r:-0.489) was found between the pre-LC BChE values and the glucose value of obese individuals, and there was no statistically significant correlation between these two parameters in the same group measured after LC (p>0.05). In the group of non-obese individuals, no statistically significant correlation was found between the parameters examined before and after LC (p>0.05). (Figure 2)

Table 1. Demographic information of the study group consisting of cholelithiasis patients

	Cholelithiasis (N=31)
Age (years; X ±S)	47.8±2.3
Gender (Male/Famale)	8/23
Body mass index (kg/m^2 ;X ±S)	30.1±4.3

BMI: Body-Mass Index

Table 2. Comparison of biochemical parameters of the study group before and after cholecystectomy

Tests	Before cholecystectomy (n=31)	After cholecystectomy (n=31)	p value
BChE (U/L; x̄ ±S)	7996±1698	7578±1600	0.215
BChE/HDL (U/mg; x̄ ±S)	17.75±5.36	17.48±6.72	0.299
Cholesterol (mg/dL; x̄ ±S)	208±38	193±34	0.015*
Triglyceride (mg/dL; Median, min-max	130 (100-156)	157 (97-220)	0.289
HDL (mg/dL; Median, min-max)	46 (41-52)	45 (41-53)	0.315
LDL (mg/dL; x̄ ±S)	129±35	113±29	0.010*
VLDL (mg/dL; x̄ ±S)	30±18	35±20,4	0.289
Fasting blood glucose (mg/dL; Median, min-max	98 (93-113)	100 (88-114)	0.651
Creatinin (mg/dL; x̄ ±S)	0.82±0.14	0.84±0.15	0.580
Protein (mg/dL; x̄ ±S)	7.3±0.4	7.4±0.4	0.155
Albumin (mg/dL; x̄ ±S)	4.3±0.3	4.4±0.3	0.101

Parametric data are expressed as the mean (x̄) ± standard deviation (S), while non-parametric data are expressed as the median (Min-Max). BChE, Butyrylcholinesterase; HDL, high- density lipoprotein; LDL, low- density lipoprotein; VLDL, very low- density lipoprotein. * p < 0.05.

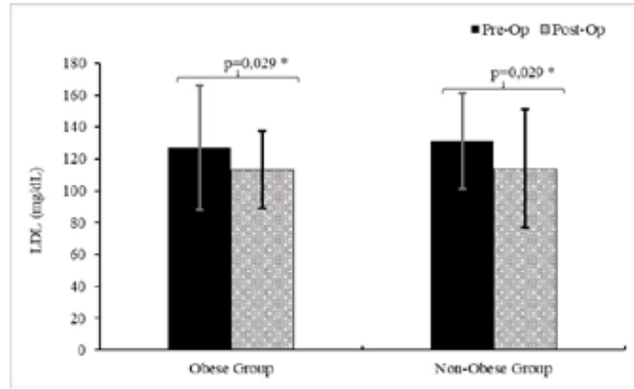

Figure 1. Pre and post-operative LDL levels of patients in obese and non-obese groups

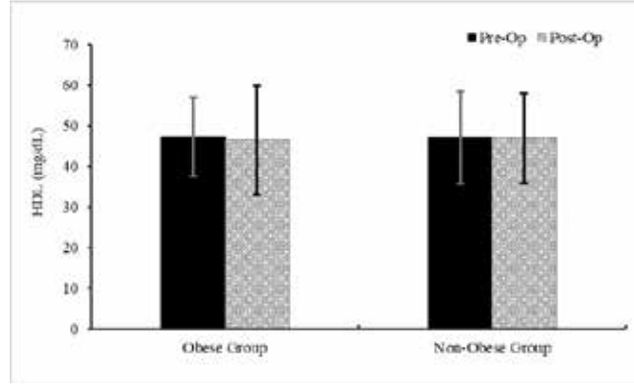

Figure 2. Pre and post-operative HDL levels of patients in obese and non-obese groups

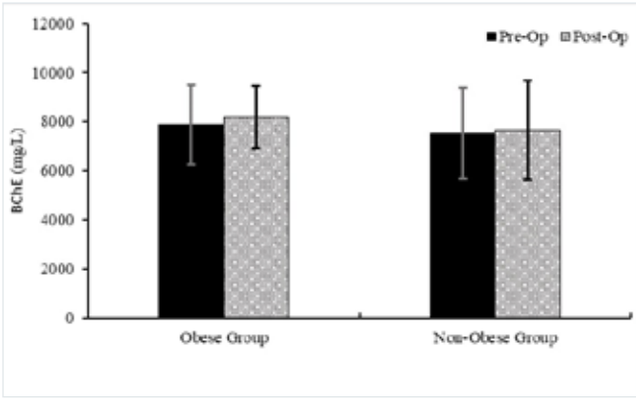

Figure 3. Pre and post-operative BChE levels of patients in obese and non-obese groups

Discussion

In our study, it was investigated whether there is a change in serum lipid profile and BChE enzyme levels in patients with cholelithiasis after LC. As a result of the data we obtained, it was found that plasma total cholesterol and LDL levels significantly decreased after LC in cholelithiasis patients ($p < 0.05$), while there was no significant difference in VLDL, HDL and TG levels. According to our knowledge, although there is no study investigating the change in the BChE enzyme level using LC application in patients with cholelithiasis, there are various studies investigating lipid profiles after LK in cholelithiasis patients [22]. In two different studies, Al-Kataan has found a significant decrease in total cholesterol, TG and LDL levels 1 month after LC application in 60 cholelithiasis patients, while a significant increase has been found in HDL levels [23]. In a study by Gill and Gupta, it has been determined that while a significant decrease in total cholesterol and TG levels was found in 50 patients with gallbladder stone, 1 month after cholecystectomy, HDL levels increased significantly. In addition, no significant difference has been observed in LDL and VLDL levels one month after the operation [24]. Considering these studies, although there are significant changes in lipid profile values after LC in cholelithiasis patients, there are some contradictory results. In this contradiction, the number of individuals examined in the study and the time elapsed after the operation may play an important role. Therefore, studies that will examine the changes in lipid levels after LC in a longer period and in more individuals are required. Researchers focused more on the changes in lipid levels after LC. However, there have not been enough studies on the mechanism of these changes.

The BChE enzyme is also synthesized by extrahepatic tissues, mainly the liver. BChE is especially secreted from adipose tissue outside the liver and its secretion is related to the fat mass. Although BChE is mostly used as a liver function test, it is an enzyme that is also effective in the conversion of VLDL to LDL [20]. In addition, the study has revealed a significantly positive correlation between serum BChE enzyme levels and LDL, total cholesterol and triglyceride levels [16]. Similarly, Alacantara et al. also have found a strong positive correlation between BChE and TG, total cholesterol and LDL levels. In addition, a negative correlation between BChE and HDL levels has been analyzed [13]. In addition, in another study, a positive correlation has been found between BChE enzyme and LDL in patients with hepatocellular carcinoma [23]. Based on these studies, it is understood that there is a significant relationship between BChE enzyme levels and serum lipid levels, especially LDL. Therefore, in our study, we also determined the activity of this enzyme before and after LC in order to examine the relationship between BChE enzyme levels and serum lipid levels. As a result of the data we obtained, although a statistically significant value was not reached, there was a moderate decrease in BChE enzyme activity after LC (7578 ± 1600 U / L) compared to before LC (7996 ± 1698 U / L). Although a significant decrease in LDL level after the operation suggests that there may be a correlation between these two parameters, no significant correlation was found between BChE and any parameter before and after LC in our analyzes.

In addition to the BChE enzyme, our study is used to evaluate serum albumin and total protein levels, the liver synthesis capacity [24]. Failure to detect a significant difference after LC in terms of all 3 parameters in our study may indicate that there is no problem with the postoperative liver synthesis capacity in patients. In addition to all these, a history of cardiovascular disease was determined as an exclusion criterion when creating the patient group in our study. However, based on the study by Rejendra et al [25] together with the lipid profile, the BChE/HDL ratio was evaluated as a "complementary risk factor" for cardiovascular disease in order to assess the risk of unknown cardiovascular disease. It was determined that there was no statistically significant difference in BChE / HDL ratio in terms of values obtained before and after LC. Finally, in our study, the patient group was classified into two groups as obese and non-obese individuals according to their BMI values. When evaluated statistically in terms of parameters measured before and after LC, it was observed that only LDL levels decreased significantly in both obese and non-obese groups after LC, while there was no significant difference in terms of any other parameter.

In conclusion, the fact that liver function tests such as BChE, albumin and total protein did not change significantly 6 months after LC, and that the lipid profile showed a significant decrease in total cholesterol and LDL levels support the idea that LC is a reliable and advantageous application in patients with cholelithiasis. However, our findings should be supported by comprehensive studies with a large number of people.

Limitations of the Study:

First of all, our study is planned as a pilot study for other detailed studies that we will conduct. Although our study was well planned, more significant results can be obtained with the data obtained from examining different parameters by increasing the number of patients. Secondly, by determining gene polymorphisms that are thought to be effective in BChE enzyme activity, the changes of BChE activity and lipid profiles can be examined at genetic level in a similar study group. In addition, when the study group was formed, the patients' post-operative drug use should be determined as an exclusion criterion or their drug use should be controlled in detail. In addition, in order to better elucidate the effects of the currently popular cholecystectomy methods, the study should be examined in both open and LC forms. Thus, the effects of the two surgical methods can be evaluated together.

Scientific Responsibility Statement
The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest
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